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Modern vaccines/adjuvants formulation

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The Modern Vaccines/Adjuvants Formulation meeting aims to fill a critical gap in current vaccine development efforts by bringing together formulation scientists and immunologists to emphasize the importance of rational formulation design in order to optimize vaccine and adjuvant bioactivity, safety, and manufacturability. Session 6 on Vaccine and Adjuvant Formulation and Production provided three examples of this theme, with speakers emphasizing the need for extensive physicochemical characterization of adjuvant-antigen interactions, the rational formulation design of a $CD8^+$ T cell-inducing adjuvant based on immunological principles, and the development and production of a rabies vaccine by a developing country manufacturer. Throughout the session, the practical importance of sound formulation and manufacturing design accompanied by analytical characterization was highlighted.

Characterization of Vaccine Formulations with Adjuvants

The complexity of the interactions between vaccine antigens and adjuvants, and the resulting effects on vaccine efficacy, are often underappreciated.[1] Dr Lakshmi Khandke from Pfizer Vaccine Research in Pearl River, New York, USA underscored this topic along with its relevant corollaries such as dose, delivery route, and single vial or two-vial presentation. When considering antigen-adjuvant compatibility, there are many attributes that should be monitored including appearance, density, viscosity, pH, particle size, surface charge, adsorption and/or binding, chemical or physical degradation, and length of stability after mixing. The strength of interaction between antigen and adjuvant, such as in the case of adsorption to aluminum salts, and the low doses of antigen and adjuvant involved, can make characterization challenging. However, applying different stress conditions such as exposure to light, elevated temperatures, freeze/thaw cycles, or agitation can facilitate stability comparisons and prediction of shelf-life.

Case studies applying the principles listed above involved (1) the formulation of a TLR9 agonist CpG oligodeoxynucleotide with aluminum oxyhydroxide and vaccine antigen, where the pH, ratio of CpG to aluminum, and choice of antigen differentially affected the binding of CpG or antigen to the aluminum; (2) selection of the optimal pH for an adjuvant-antigen combination when one component was less stable at lower pH values whereas the other component was less stable at higher pH values, with ion exchange HPLC indicating the length of stability of the antigen after mixing with the adjuvant; and (3) employment of fluorescence spectroscopy, circular dichroism, and differential scanning calorimetry to characterize the effects on antigen secondary and tertiary structure, and thermal stability, after adsorption to aluminum. Dr Khandke indicated some of the more useful analytical techniques for characterization of vaccines containing adjuvants, including electron microscopy, dynamic light scattering, isothermal titration calorimetry and differential scanning calorimetry, traditional intrinsic and front face fluorescence spectroscopy, and zeta potential measurement. Due to the complexity of many of the methods, they are better employed as investigative tools to understand interactions rather than as quality control methods.

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Engineering a $CD8^+$ T-cell-inducing Vaccine Adjuvant through Rational Formulation Design

Dr Dennis Christensen from Statens Serum Institut in Copenhangen, Denmark updated attendees on the latest developments in the cationic adjuvant formulation (CAF) series, focusing on CAF09 and the modifications necessary to facilitate induction of $CD8^+$ T cells via cross presentation after subcutaneous immunization. CAF09 consists of three main components: Dimethyldioctadecylammonium (DDA), monomycoloyl glycerol synthetic analog (C_{32} MMG), and polyinosinic:polycytidylic acid [poly (I:C)]. Since DDA has no hydrogen bonding capability, the addition of C_{32} MMG helps stabilize the DDA vesicles through interaction with water. Moreover, previous work by Christensen and collaborators have shown that changing the charge or the level of saturation in the lipid chain of DDA has detrimental effects on Th1-type adjuvant activity.[2,3] MMG is one of the principal components responsible for the dendritic cell stimulating activity of mycobacterial cell wall.[4] Poly(I:C) was chosen as an additional component of the formulation since TLR3 is highly expressed on cross-presentation-capable dendritic cells.[5]

Initial work by Christensen demonstrated that CAF09 elicited ovalbumin (OVA)-specific $CD8^+$ T cells after intraperitoneal (i.p.) or intranasal (i.n.) administration but not subcutaneous (s.c.) immunization. With the goal of generating a $CD8^+$ T cell response following s.c. administration, CAF09 characteristics were rationally modified to promote lymph node targeting. Indeed, such modifications of CAF09 resulted in enhanced $CD8^+$ T cell response after s.c. administration with OVA in mice. The key message from the above work is that adjuvant formulations can be rationally designed if formulation scientists and immunologists can understand and communicate to each other the critical parameters necessary to achieve a targeted and enhanced immune response. Continued development of CAF09 is planned to result in phase I clinical evaluation in the near future.

Progress to Rabies Vaccine Production in Nepal

The burden of rabies in Nepal was presented by Dr Ganesh Raj Pant from the Rabies Vaccine Production Laboratory (RVPL) in Kathmandu as background for the animal and human vaccine development and production activities performed by his lab. Rabies is endemic in Nepal, accounting for some 100–200 deaths annually (although this number may be a severe underestimation) and especially affects children.[6] Many breeds of livestock also suffer a heavy burden from the disease. Veterinary and human vaccine development and production activities have been ongoing at RVPL since 1970 and 1982, respectively, beginning with naturally derived nerve or brain tissue-based vaccine composition. In 2002–2006, a cell culture-based veterinary vaccine was developed and then marketed as NeJaRab, with 50 000 doses produced annually and a total of 35 separate lots to date. Concurrently, RVPL has been developing a Vero cell culture-based human vaccine production process. A series of quality control tests are employed to characterize the vaccine, such as virus titration, inactivation test, sterility, pH, toxicity and potency in mice, and safety in dogs. The current production process results in vaccine potency of 3.8 IU/dose, well above the WHO-recommended minimum standard of 2.5 IU/dose. The current development stage involves producing three consistency batches, after which clinical testing will be initiated. The accomplishments of RVPL in successfully developing and producing veterinary and human rabies vaccines serves as a model for other developing countries. Continued support by the international community is necessary to encourage ongoing local vaccine development and production.

Summary

This session provided a unique glimpse at the diverse spectrum of players in vaccine and adjuvant development: a large multinational pharmaceutical company, an established European health research institute, and a developing country manufacturer. Despite the great differences in the scope, products, and facilities available to each institute, the presentations in this session emphasized that there are a set of common principles which must underlie all vaccine and adjuvant development. These principles include rational formulation design, thorough characterization (physicochemical, biological, safety) of products produced, and manufacturability. These topics must always be of paramount interest for all vaccine and adjuvant developers if effective and manufacturable products are to be developed to solve unmet needs.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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